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"DESIGN, DEVELOPMENT AND EVALUATION OF LIPOSOMES CONTAINING ANTICANCER DRUG"

A SYNOPSIS SUBMITTED FOR THE REGISTRATION OF *DEGREE OF DOCTOR OF PHILOSOPHY*

In the Faculty of Pharmaceutical Sciences & Technology

Submitted

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INDEX

SR.NO.	CONTENT	PAGE NO
01	ABSTRACT OF PROPOSED WORK PLAN	04
02	INTRODUCTION	05
03	REVIEW OF LITERATURE AND DEVELOPMENT IN THE SUBJECT	07
04	OBJECTIVES OF RESEARCH	12
05	MATERIALS AND METHODOLOGY TO BE ADOPTED	13
06	IMPORTANCE OF STUDY	15
07	PROPOSED WORK PLAN	16
08	REFERENCES	17

1. Abstract of proposed work plan/ problem:

Cancer continues to be a major life-threatening disease. The most limiting factors in treatment of cancer are mutation and drug resistance in the cancer cells, poor selectivity and immunosuppressive action, side effect of the anticancer drugs, and low penetration in solid tumor. Liposomes have raised considerable interest as drug carriers in cancer chemotherapy because of their ability to alter the biodistribution by selectively targeting the drug to the tumor and control the release rate of encapsulated drugs. Such formulation and drug targeting strategies enhance the effectiveness of anticancer chemotherapy and reduce at the same time the risk of toxic side-effects. In spite of desirable feature of liposome as carrier, they suffer from disadvantages such as their rapid clearance from systemic circulation leading to short duration of action, less stability and drug leakage resulting into loss of drug before it reaches the target site. Such draw backs can be easily overcome by coating the liposome with polyethylene glycol. Based on the review of articles on liposomes we found that it's a need of adding antioxidant agent like glutathione, glutamic acid to overcome peripheral neuropathy of anticancer formulation. Anticancer drug-loaded liposomes will be prepared by reverse phase evaporation or sonication method. Such prepared drug loaded liposomes will be optimized and evaluated for particle size, zeta potential, entrapment efficacy, PXRD, DSC, TEM, in-vitro drug release, in vivo pharmacokinetic study, in vitro anticancer cell line study and stability studies.

2. Introduction:

Liposomes are small artificial vesicles of spherical shape that can be fashioned from cholesterol and natural nontoxic phospholipids. Due to the size and their hydrophobic and hydrophilic character (besides biocompatibility), liposomes are highly capable systems for drug delivery. Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation. Furthermore, the choice of bilayer components determines the 'rigidity' or 'fluidity' and the charge of the bilayer. Because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues, liposomes have increased importance, both as an investigational systems well as a commercial drug delivery system. Many studies have been conducted on liposomes with the goal of decreasing drug toxicity and/ or targeting specific cells**.** Numerous different liposome formulations of various anticancer agents were shown to be less toxic than the free drug. Anthracyclines are drugs which stop the growth of dividing cells by intercalating into the DNA and thus, kill mainly rapidly dividing cells. These cells are not only in tumors but are also in hair, gastrointestinal mucosa, and blood cells; therefore, this class of drugs is very toxic. In addition to the above-mentioned acute toxicities, its dosage is limited by its increasing cardio toxicity. Numerous diverse formulations were tried. In most cases, the toxicity was reduced to about 50%. These include both acute and chronic toxicities because liposome encapsulation reduces the delivery of the drug molecules towards those tissues. For the same reason, the efficiency was in many cases compromised due to the reduced bioavailability of the drug, especially if the tumor was not phagocytic or located in the organs of mononuclear phagocytic system. In some cases, such as systemic lymphoma, the effect of liposome encapsulation showed enhanced efficacy due to the continued release effect, i.e., longer presence of therapeutic concentrations in the circulation, while in several other cases, the sequestration of the drug into tissues of mononuclear phagocytic system actually reduced its efficacy. Liposomes are thus the promising novel delivery carrier systems for anticancer drugs to overcome their side effects, to improve the target specificity, to enhance the pharmacokinetic parameters etc.

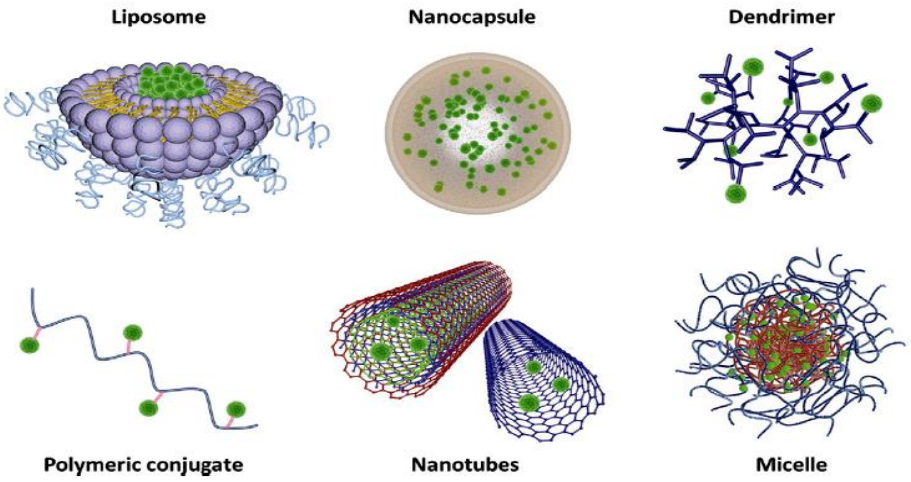

Fig: Different novel drug delivery carrier

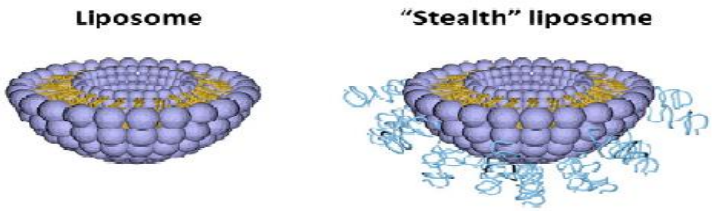

Fig: Pegylated liposomes

3. Review of Literature and Development in the subject (Previous work done in the relevant area):

1) Abolfazl Akbarzadeh, Rogaie Rezaei-Sadabady, Soodabeh Davaran, Liposomes, sphere-shaped vesicles consisting of one or more phospholipid bilayers were first described in the mid-60s. Today, they are a very useful reproduction, reagent, and tool in various scientific disciplines, including mathematics and theoretical physics, biophysics, chemistry, colloid science, biochemistry, and biology. Since then liposomes have made their way to the market. Among several talented new drug delivery systems, liposomes characterize an advanced technology to deliver active molecules to the site of action, and at present, several formulations are in clinical use. Liposomes with modified surfaces have also been developed using several molecules, such as glycolipids or sialic acid.

2) Yogita P. Patil, Sameer Jadhav, Liposomes are bilayer vesicles which have found use, among other applications as drug delivery vehicles. Conventional techniques for liposome preparation and size reduction remain popular as these are simple to implement and do not require sophisticated equipment. However, issues related to scale-up for industrial production and scale-down for point-of-care applications have motivated improvements to conventional processes and have also led to the development of novel routes to liposome formation. In this article, these modified and new methods for liposome preparation have been reviewed and classified with the objective of updating the reader to recent developments in liposome production technology.

3) **Hongyan Wei, Juan Song , Hao Li,** The aim of the present study was to investigate the effect of various transmembrane ammonium salt gradients and different lipid composition on the loading efficiency of liposomal formulations of irinotecan hydrochloride (CPT-11), their behavior in vivo and cytotoxicity. Among ammonium salts studied, ammonium sulfate was successfully used to load CPT-11 into liposomes with the highest encapsulation efficiency. Subsequently, liposomal CPT-11 with different lipid composition was prepared by ammonium sulfate gradient method.

4) J. Allen Zhang, Gopal Anyarambhatla, Lan Ma, Taxol is a marketed product for the treatment of ovarian, breast, non-small cell lung cancer and AIDS-related Kaposi's sarcoma. It is thus far one of the most effective anticancer drugs available on the market. However,

paclitaxel is only sparingly soluble in water and therefore, intravenous administration depends on the use of the non-ionic surfactant Cremophorw EL (polyethoxylated castor oil) to achieve a clinically relevant concentrated solution. Unfortunately, Cremophorw EL increases toxicity and leads to hypersensitivity reactions in certain individuals. We have developed a well characterized novel lyophilized liposome-based paclitaxel (LEP-ETU) formulation that is sterile, stable and easy-to-use. The mean particle size of the liposomes is about 150 nm before and after lyophilization, and the drug entrapment efficiency is greater than 90%. Stability data indicated that the lyophilized LEP-ETU was physically and chemically stable for at least 12months at 2–8 and 25 8C. Moreover, the formulation can be diluted to about 0.25 mg/ml without drug precipitation or change in particle size. In vitro drug release study in phosphate-buffered saline (PBS, pH 7.4) showed that less than 6% of the entrapped paclitaxel was released after 120 h, indicating that the drug is highly stable in an entrapped form at physiologic temperature.

5) **Chuang Yang, Zhong Xue Fu**, Oxaliplatin is effective for the treatment of advanced colorectal cancer; however, its application is restricted due to its dose limiting toxicity. Liposomes are sphere shaped vesicles consisting of one or more phospholipid bilayers. Liposomes as drug carriers are characterized by delayed release, lesion targeting and may be used as a drug delivery system to decrease the side effects of cytotoxic drugs. Active targeting modification of liposomes may change the biological distribution of the anticancer agents, reduce or reverse multidrug resistance of tumor cells and enhance the effects of anticancer therapy. Based on the characteristics mentioned above, the aim of the present review was to demonstrate that polyethylene glycol liposomes containing oxaliplatin may offer advantages for the treatment of colorectal cancer in clinical practice.

6) Preety Panwar, Bhumika Pandey, P C Lakhera, The purpose of the present study was to formulate effective and controlled release albendazole liposomal formulations. Albendazole, a hydrophobic drug used for the treatment of hydatid cysts, was encapsulated in nanosize liposomes. Rapid evaporation method was used for the preparation of albendazole encapsulated conventional and PEGylated liposomes consisting of egg phosphatidylcholine (PC) and cholesterol (CH) in the molar ratios of (6:4) and PC:CH: polyethylene glycol (PEG) (5:4:1), respectively. In this study, PEGylated and conventional liposomes containing albendazole were prepared and their characteristics, such as particle size, encapsulation

efficiency and *in vitro* drug release were investigated. The drug encapsulation efficiency of Pegylated and conventional liposomes was 81% and 72%, respectively. The biophysical characterization of both conventional and PEG coated liposomes were done by transmission electron microscopy and UV vis spectrophotometry. Efforts were made to study *in vitro* release of albendazole. The drug release rate showed decrease in albendazole release in descending order: free albendazole, albendazole loaded conventional liposomes and least with albendazole loaded PEG liposomes. Biologically relevant vesicles were prepared and *in vitro* release of liposome entrapped albendazole was determined.

7) Eskandar Moghimipour, Somayeh Handali, Liposomes are polymeric nanoparticles used for drug delivery due to their unique properties. Lipisomes can encapsulate both hydrophobic and hydrophilic drug. Liposomes deliver the drugs into cells by fusion or endocytosis mechanisms. In the last few decades, liposomes have been considered as ideal models for mimic biological membranes and also they are suitable carriers for drugs, diagnostics, vaccines, and other bioactive agents. Drugs can be distributed non-specifically throughout the body, lead to death of normal and malignant cells. Entrapment of drugs into liposomes results in increasing circulation life time, protection from the metabolic degradation, enhancement of deposition in the infected tissues and decreased uptake in the kidney, myocardium and brain.

8) Janina Gabrielska, Małgorzata Korzeniowska, Aneta Wojdyło, The use of liposomes and erythrocytes membrane as a real membrane models to evaluate the potential benefits of several plants extracts and two flavones in relation to lipid peroxidation. The antioxidant behaviour of the plant extracts from pine (Pinus silvestris L), hawthorn (Crataegus oxyacantha L, two extracts: from hawthorn's leaves-l and bark-b), evening primrose (Oenothena paradoxa Hudziok – three extracts differ in procyanidins content P1, P2 and P3) and rosemary (Rosmarinus officinalis, as a standard for extracts) and flavones of baicalin and rutin have been studied. The results obtained showed that the studied extracts exhibited differentiated, dose-dependent antioxidant activity against phospatidylcholine liposomes (rosemary>pine≈hawthorn-l>hawthorn-b≈P1≈P2≈P3; statistically significant differences were observed between the extracts at $p \leq 0.05$) and erythrocyte membranes (rosemary≥hawthorn-b≈hawthorn-l>P1≈pine>P2≈P3) when the oxidation was induced by UV-C radiation. They also reduce the oxidation of liposomes and erythrocyte membrane when its oxidation was

induced by 2, 2'-azobis (2-amidinopropane) dihydrochloride (pine≥P1≈P2≈rosemary≈P3 in the case of liposomes and rosemary>> pine≥P1≈P2≥P3 in a case of erythrocyte). Moreover, the results of the study show that baicalin is characterized by high inhibition ability towards liposome PC peroxidation, as well as towards erythrocyte ghosts, when oxidation was initiated by UV radiation. However, at the same experimental conditions, the inhibitory capacity of rutin was about 7-8 times weaker. The presence of cholesterol in liposome membrane decreased the level of membrane peroxidation but do not influenced on the antioxidant activity of hawthorn extract.

9) Haresh Patel, Hiren Patel, Nitesh Patel, Amit Gupta, Vinblastine sulfate loaded liposomes were prepared with an aim to improve stability, reduce drug leakage during systemic circulation, and increase intracellular uptake. Liposomes were prepared by the thin-film hydration method, followed by coating with calcium phosphate, using the sequential addition approach. Prepared formulations were characterized for size, zeta potential, drug-entrapment efficiency, morphology by transmission electron microscopy (TEM). Effect of formulation variables, such as drug: lipid ratio as well as nature and volume of hydration media, were found to affect drug entrapment. Size, zeta potential, and TEM images confirmed that the liposomes were effectively coated with calcium phosphate.

10) Abdalrahim FA Aisha, Amin Malik Shah Abdul Majid and Zhari Ismail1, *O. stamineus* is a medicinal herb with remarkable pharmacological properties. However, poor solubility of the active principles limits its medicinal value. This study sought to prepare nano liposomes of OS ethanolic extract in unpurified soybean phospholipids in order to improve its solubility and permeability. OS liposomes were prepared by the conventional film method, and were characterized for solubility, entrapment efficiency, Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), particle size and zeta potential, release, absorption in everted rat intestinal sacs, and DPPH scavenging effect.

11) Olga Gortzi , Stavros Lalas , Ioanna Chinou John Tsaknis, The antioxidant and antimicrobial activity of methanol or dichloromethane extracts of *O. dictamnus*, produced from wild and organic cultivated specimens, were determined. The Rancimat and malondialdehyde (MDA) by HPLC methods were used to measure the antioxidant action, in comparison with that of the common commercial antioxidants butylated hydroxytoluene (BHT) and α-tocopherol. The extracts that presented high antioxidant activity were

encapsulated in liposomes and their antioxidant action was again estimated using differential scanning calorimetry (DSC). Thermaloxidative decomposition of the samples (pure liposomes and encapsulated extracts) and the modification of the main transition temperature for the lipid mixture and the splitting of the calorimetric peak in the presence of the antioxidants were also studied by the DSC method. All extracts showed antioxidant and antimicrobial activities. Their action proved superior to α-tocopherol. The methanol extract of organic cultivated *O. dictamnus* (240 ppm) showed higher activity than butylated hydroxytoluene. After encapsulation in liposomes the antioxidant as well as antimicrobial activities proved to be higher than those of the same extracts in pure form.

4. Objectives of Research/ Proposed Hypothesis:

Aim: Design, Development and Evaluation of Liposomes Containing Anticancer Drug

Objectives of the Research Work:

- To formulate, optimize and characterize anticancer drug-loaded liposomes.
- To improve the efficacy, drug loading capacity and biocompatibility of anticancer drug by formulating in liposomes.
- To minimize the toxicity and increase the stability of anticancer drug by encapsulating in liposomal system.
- To evaluate anticancer potential of formulation by in-vitro cell culture study.
- To evaluate in-vivo bioavailability of drug from formulation in comparison with pure drug in experimental animal.
- To study the tissue distribution of drug from formulation in comparison with pure drug in experimental animal.

5. Materials and Methodology to be adopted:

1. **Literature review**
2. **Selection and Collection of anticancer drug & excipients**
    1. Selection of anticancer drug: e.g. Oxaliplatin or Vincristine or Paclitaxel or Etoposide etc.
    2. Solvents: Diethyl ether AR, Chloroform AR etc.
    3. Chemicals: 10% sucrose, 5% Dextrose and 0.9% saline etc.
    4. Lipids: Hydrogenated Phosphotidilcholine from Soyabean, Dipalmitoylphosphocholine, Polyethelene glycol-2000- distearoylphosphoethanolamine and Cholesterol (CH) etc.

3. **Experimental work**
    - **A. Characterization of drug and excipients**
        1. **Spectroscopic studies**
        2. **Solubility studies**
        3. **Crystallinity studies**
    - **B. Compatibility studies**
4. **Formulation development**

    Techniques used in preparation of Liposomes:

    A) Liposomes can be prepared by number of methods which are as follows:

    1. Lipids film hydration technique.
    2. Modified heating method.
    3. Reverse phase evaporation technique.
    4. Sonication viz.

    B) Optimization of lyophilized liposomes.

5. **Characterization of drug-loaded liposomes**

- Particle size and zeta potential
- Encapsulation efficiency
- Surface morphology by Transmission electron microscopy (TEM)
- Fourier transform infrared spectroscopy
- PXRD study

- Differential scanning colorimetry
- In vitro drug release etc.

6. **In vitro anticancer cell line study**
7. **In vivo pharmacokinetic study**
8. **Stability studies.**

6. Importance of study/ Society application:

- **International status:** Nanotechnology is a rapidly evolving domain, as it answers to various issues associated with conventional drug delivery. This is very true in diseases like cancer where there are many challenges. Worldwide there is increasing number of cancer patients, consequently anticancer drug use also increased. Most of the studies in last few years with liposomes showed a promising result and accordingly there is increasing number of patents on novel nano drug delivery in chemotherapy of cancer.
- **National status:** Being a second largest populated country in the world, India is facing a many more challenges in cancer therapy. Increased number of cancer patients in India due to smoking, sedentary life style, poor living standard, pollution, lack of awareness on cancers early signs and symptoms etc. A big issue in India is lack of availability of most effective, safe and economic drug delivery for cancer chemotherapy. Advanced liposomes preparation have the potential to overcome the challenges related to cancer chemotherapy. There is huge scope in India to develop a drug delivery with improved efficacy, biocompatibility, biodistribution and minimized toxicity in cancer chemotherapy.

►Significance of the study:

Liposomes are among the first nanomolecular drug delivery systems to demonstrate the increased delivery of small molecular weight anticancer drugs to solid tumors by altering the biodistribution of associated drugs.To improve the efficacy of drug by targeted drug delivery in order to reduces toxicity metastasis of colorectal cancer, increase bioavailability, blood circulation time since PEG decreases the recognition of liposomes by the mononuclear phagocyte system thereby exhibiting prolong half-life. Mostly reduced peripheral neuropathy of anticancer formulation by adding glutathione like antioxidant agent in there preparation.

Liposomes as a novel type of drug carrier,

- Exhibit good targeting properties
- Slow releasing potential
- High stability (freeze dried)
- Low toxicity with surface modification.

7. Proposed work Plan/ Formulation and Structure of Study:

Year-wise Plan of work and targets to be achieved:

Sr. No.	**Expected target to be achieved**	**1-6 months**	**7-10 months**	**11-22 months**	**22--27 months**	**28- 33 months**	**34-36 Month**
01	Literature survey	Literature survey					
02	Selection and collection of drug and excipients	Selection and collection of drug and excipients					
03	Formulation, development and optimization	Formulation, development and optimization					
04	Evaluation of Liposomes	Evaluation of Liposomes					
05	In vitro and in vivo study	In vitro and in vivo study					
06	Statistical Analysis, Data Interpretation and Conclusions	Statistical Analysis, Data Interpretation and Conclusions					
07	Publishing Research and submitting data	Publishing Research and submitting data					

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